Mechanism of sodium and water retention in rats with experimental heart failure

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Mechanism of sodium and water retention in rats with experimental heart failure. High output heart failure was produced in male rats by placing an aorta-to-vena cava (A-V) fistula distal to the renal arteries. Rats with an A-V fistula developed sustained salt retention, peripheral edema and ascites within 10 to 20 days after the operation. The urinary excretion of sodium was significantly lower in the A-V rats as compared to the control rats. No difference in total GFR, intrarenal distribution of filtration (Hanssen technique), PAH clearance or filtration fraction was found between the two groups. Also, fractional and absolute sodium reabsorption by the proximal tubule were not different for the two groups. Moreover, after acute saline loading, similar decreases in proximal tubular reabsorption occurred in controls and rats with an A-V fistula. However, A-V rats reabsorbed significantly more sodium and water by the loop of Henle than did the controls, both before and after volume expansion. The enhanced rate of reabsorption by the loop of Henle is thought to be one important factor which causes chronic salt retention and edema formation in A-V rats.

Mécanisme de la rétention d'eau et de sodium chez les rats atteints d'insuffisance cardiaque expérimentale. Une insuffisance cardiaque à débit élevé est produite chez des rats mâles par la mise en place d'une fistule aorto-cave distale par rapport aux artères rénales. Chez ces rats apparaissent dans les dix jours qui suivent l'intervention, une rétention d'eau et de sel prolongée, des oedemes périphériques et une ascite. L'excrétion urinaire du sodium est significativement inférieure chez les rats porteurs de fistules pas comparaison avec des contrôles. Aucume différence autre les deux groupes n'a été trouvée en ce qui concerne le débit global de filtration glomérulaire, la distribution intra-rénale des filtrations (technique de Hanssen), la clearance du PAH et la fraction filtrée. De plus la réabsorption fractionnelle et absolve du sodium pas le tube proximal n'était pas différente dans les deux groupes. Enfin, après une surcharge en sel aiguë, une inhibition similaire de le réabsorption tubulaire proximale a été observée dans les deux groupes. Cependant, les rats porteurs de fistule réabsorbent significativement plus de sodium et d'eau dans l'anse de Henle que ne le font les contrôles, ceci avant et après expansion. L'augmentation du débit de réabsorption par l'anse de Henle est probablement un facteur important de la rétention chronique de sel et de la formation d'oedemes chez les rats porteurs d'une fistule.

Experimentally induced low or high output heart failure in dogs has been widely used by several investigators to study the pathophysiology of chronic sodium retention and edema [1–6]. In contrast, there is no experimental information available from sustained salt retention in the rat. The lack of a predictable model of heart failure in the rat may explain the paucity of data on renal tubular function during the course of cardiac decompensation and chronic sodium retention. Recently, we have described a method of producing high output heart failure in the rat with subsequent formation of edema by placing an aorta-to-vena cava (A-V) fistula distal to the renal arteries [7]. Initial experiments revealed that the development of chronic sodium retention and edema formation in this preparation could not be correlated with depression of total glomerular filtration rate (GFR) or renal plasma flow. Thus, the low renal sodium excretion of rats with an A-V fistula could be due either to redistribution of glomerular filtrate [8], or to an enhanced rate of sodium reabsorption along the nephron. The site of altered sodium reabsorption along the nephron in rats with an A-V fistula is not well understood. Thus, the present study was undertaken to elucidate the renal mechanism responsible for the limited ability of rats with an A-V fistula and edema to excrete sodium. Micropuncture experiments were performed to measure the rate of sodium and water reabsorption by superficial proximal tubules and short loops of Henle in normal rats and those with an A-V fistula. Also, the effect of acute saline loading on proximal sodium reabsorption and urinary excretion was investigated in these same rats. Intrarenal distribution of filtrate was assessed using the ^{14}C-ferrocyanide method. The data provide evidence that an enhanced rate of sodium and water reabsorption by the

loop of Henle provides at least one important mechanism for the sustained salt and fluid retention exhibited by rats with an A-V fistula.

Methods

Male Sprague-Dawley rats weighing 250 to 300 g were anesthetized with pentobarbital sodium (40 mg/kg of body wt). Under semisterile conditions a 0.9 to 1.5 mm elliptical A-V fistula was placed approximately 10 mm distal to the origin of the renal arteries. This was accomplished by dissecting the vena cava and the aorta from the surrounding tissues, but not from each other. Two miniature vessels clamps (MET, Europavertrieb F. L. Fischer, 78 Freiburg, W. Germany) were placed around both vessels 5 to 10 mm apart, and a 1 to 1.5 mm opening was cut into the lateral wall of the vena cava. The common wall between the vena cava and the aorta was grasped through the incision by a forceps and the fistula was established between the vessels with small scissors. A continuous perlon suture No. 6 (MET) was used to close the defect of the vena cava. After the vessel clamps were released arterial blood could be seen vigorously entering the vena cava through the fistula and mixing with the darker venous blood. Usually the time of occlusion of both vessels did not exceed 10 minutes. No heparin was administered. Fourteen control animals were operated on in identical fashion but a fistula was not established. All the animals were placed in metabolic balance cages to measure daily sodium excretion. The rats were fed a synthetic diet and water was allowed *ad libitum* (see reference 9 for diet composition). Rats with an A-V fistula developed sustained salt retention and peripheral edema within 10 to 20 days after the operation. Only rats having clearly demonstrable ascites and peripheral edema were used for the micropuncture experiments. The mean diameter of the circular A-V fistula in those rats that exhibited sustained sodium retention and edema formation was 1.15 ± 0.04 mm. The size of the orifice was estimated with the aid of an eyepiece micrometer.

Seventeen to 27 days after the operation, the rats were anesthetized by peritoneal injection of inactin (Promonta-Werk, Hamburg, W. Germany), 70 mg/kg of body wt, and prepared for micropuncture as previously described [10]. Care was taken to avoid the loss of ascitic fluid in the rats with an A-V fistula. In pilot experiments no change in blood pressure, renal hemodynamics or end-proximal tubular fluid/plasma (TF/P) inulin ratios had been observed after partial drainage of the ascitic fluid. All rats were pretreated with an injection of desoxycorticosterone (Percorten M, Ciba, Basel), 50 mg/kg of body wt, and 2 U of Pitressin tannate in oil on the morning of each experiment. Blood pressure was measured continuously via a heparin-filled PE 50 catheter in the left common carotid artery, connected to a strain gauge (Statham Instruments, Inc., Oxnard, Calif.). In 6 rats with an A-V fistula, blood pressure was determined simultaneously in both the left common carotid artery and the femoral artery. Rats with an A-V fistula had a consistently higher arterial blood pressure above than below the fistula, the mean difference being 38.5 ± 3.2 mm Hg. For determinations of GFR and the concentration of inulin in tubular fluid and plasma, a priming dose of 30 µCi of ^{14}C-labeled inulin carboxylic acid (New England Nuclear Corporation, Boston, Mass.) and a sustaining infusion of 1 µCi/min in isotonic saline solution were administered. During hydropenia the sustaining infusion was delivered at a rate of 0.02 ml/min. Tubule fluid samples were collected from end-proximal and early distal tubule puncture sites (for methodologic details see reference 10). The recollection technique was used exclusively during this study. The location of the puncture site was recorded by drawing a map in order that tubule fluid could be recollected from the same site after saline loading. Measurement and calculation of superficial single nephron filtration rate (SNGFR) were performed as reported in a previous study [10].

The end of the proximal convolution was located by observing the passage of lissamine green through the proximal tubule [11]. After the distal tubular fluid was collected, the tubule was filled with neoprene. Dissection and identification of puncture sites were performed according to the procedures described by Gottschalk and Mylle [12]. For calculation of reabsorption in Henle's loop,[1] only samples obtained from a puncture site 20 to 30% along the length of the distal tubule were used. Proximal and loop of Henle transit times were measured as previously described [10]. In the micropuncture study outlined above, the following experimental protocol was used. Twelve control rats and 14 A-V fistula rats with peripheral edema and ascites were investigated during continued hydropenia (rate of infusion: 0.02 ml/min) and extracellular fluid volume expansion. After two control periods and 5 to 8 proximal or distal micropuncture samples had been obtained, volume expansion was accomplished by infusing isotonic saline solution (containing appropriate amounts of ^{14}C-inulin and *para*-aminohippurate (PAH) at 0.5 ml/min until the rats had received a volume equal to 8% of their body weight. The infusion was then reduced so that it equaled or just exceeded the urine flow rate (0.1 to 0.15 ml/min). After an equilibration period of 20 minutes the micropuncture and clearance determinations were repeated.

Intrarenal distribution of glomerular filtration was measured in accordance with a technique employing an infusion of ^{14}C-ferrocyanide as described by Hanssen [13] and modified by de Rouffignac, Deiss and Bonvalet [14]. The measurements were performed in a parallel series of experiments on 10 rats with an A-V fistula and 8 control

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[1] In this study Henle's loop denotes that part of the superficial nephron which lies between the late proximal and early distal tubule puncture sites.

animals. To measure the ^{14}C-Na-ferrocyanide contained within the nephron from the glomerulus to the precipitate of nonlabeled ferrocyanide (for methodologic details see reference 14), proximal tubules were microdissected after maceration and transferred into counting vials containing 1 ml of water. Five ml of a scintillation fluid composed of two parts of toluene (with 0.4% 2,5-diphenyloxazole [PPO] and 0.005% *p*-bis[2-(5-phenyloxazolyl)] benzene [POPOP] and one part of Triton-X-100) were added to the water.

In order to determine the activity of ^{14}C-inulin, samples of tubular fluid, urine and plasma were taken up into self-filling micropipettes and transferred into counting vials containing 0.5 ml of water. Nine ml of Aquasol (New England Nuclear Corp., Boston, Mass.) was added. Mean counting efficiency for ^{14}C-inulin was 73%.

Radioassay of both ^{14}C-inulin and ^{14}C-Na-ferrocyanide samples was performed in two channels of a liquid scintillation counter (Mark I, Nuclear-Chicago Corp., Des Plaines, Ill.) to a total of at least 4000 counts. All samples had a level of radioactivity at least twice that of the background.

Sodium concentration in distal tubular fluid was measured with a dual channel microflame photometer by P. Müller (Wissenschaftlicher Gerätebau, W. Hampel, Frankfurt, W. Germany). For the measurement of sodium concentration in plasma and urine, a standard flame photometer was used (Fa. Eppendorf, Gerätebau, Hamburg, W. Germany). The PAH concentration in plasma and urine was measured by the method of Smith et al [15].

Cardiac output was estimated by thermal dilution (Herz-zeitvolumen-Meßgerät, BN 656, Fa. Hoyer, Bremen, W. Germany). Sodium excretion, glomerular filtration rate, and renal plasma flow are given for the micropunctured kidney only. No Donnan correction was used in calculation of filtered loads of sodium. For statistical analysis, either Student's *t*-test of the differences between unpaired groups (controls vs. A-V fistula rats) or the *t*-test of the mean of paired differences (volume expansion study) was used. All given deviations are ± SEM.

Results

All rats with an A-V fistula which were used in this study had sustained sodium retention. This sodium retention was identifiable clinically by the presence of pitting edema in the abdominal wall and the hind limbs, and the formation of large amounts of ascites (up to 10% of body weight). At autopsy the animals displayed hepatic congestion and pleural effusions. Fourteen sham-operated rats showed no evidence of sodium retention and there was no indication of ascitic fluid or peripheral edema. Prior to micropuncture the A-V rats had not escaped from daily sodium retention. Thus, at the time of study, these rats were in a salt-retaining stage.

In metabolic balance studies between the 10th and the 90th day after the operation, A-V fistula rats retained significantly more sodium than did the sham-operated con-

Table 1. Weekly sodium balance in sham-operated rats and A-V fistula rats[a]

	Na^+ intake *mEq/week*	Total Na^+ excretion *mEq/week*	Fractional Na^+ excretion %
A-V fistula rats ($N=14$)[b]	18.52 ± 0.57	11.80 ± 0.83	63.7
Control rats ($N=12$)	18.75 ± 0.65	17.80 ± 0.71	94.9

[a] The data were obtained between the tenth and twentieth days following the operation. Total sodium excretion (Na^+ excretion) represents the sum of urinary and fecal sodium excretion. Values are means ± SEM.

[b] Fractional Na^+ excretion (%) $= \dfrac{\text{total } Na^+ \text{excretion/week} \times 100}{Na^+ \text{ intake/week}}$

Table 2. Summary of clearance data (conditions of micropuncture)[a]

	Control rats		A-V fistula rats	
	Hydropenia ($N=12$)	Volume expansion ($N=12$)	Hydropenia ($N=14$)	Volume expansion ($N=14$)
Sodium excretion, *μEq/min/g kidney wt*	0.19 ± 0.03	9.5 ± 0.85	0.08 ± 0.01	2.35 ± 0.19
Urine flow, *μl/min/g kidney wt*	2.25 ± 0.16	53.0 ± 7.00	2.45 ± 0.45	8.05 ± 0.42
Glomerular filtration rate, *ml/min/g kidney wt*	1.12 ± 0.04	1.40 ± 0.03	1.05 ± 0.05	1.15 ± 0.04
PAH clearance, *ml/min/g kidney wt*	3.45 ± 0.24	4.30 ± 0.24	3.15 ± 0.18	3.28 ± 0.21
Filtration fraction	0.32 ± 0.023	0.32 ± 0.03	0.33 ± 0.02	0.34 ± 0.023
Mean blood pressure, *mm Hg*	121.0 ± 3.40	125.0 ± 4.30	96.50 ± 3.80	98.5 ± 4.40
Cardiac output *ml/min/100 g body wt*	37.0 ± 2.10	—	78.0 ± 6.20	—
Plasma Na^+ *mEq/liter*	145.5 ± 1.60	147.0 ± 1.65	143.5 ± 1.30	145.0 ± 1.50

[a] Values are means ± SEM.

trol animals ($P<0.001$). The results are presented in Table 1.

Clearance data. Mean results obtained under micropuncture conditions in 12 normal rats and 14 A-V fistula rats with edema and ascites are summarized in Table 2. During hydropenia A-V rats excreted significantly less sodium than did control rats ($P<0.01$). Urine flow rate was not significantly different for the two groups. No significant difference in mean total GFR and clearance of PAH was found between the two experimental groups. Arterial blood pressure measured in the carotid artery was significantly lower in all A-V fistula rats ($P<0.001$) than in control rats. However, A-V fistula rats had a markedly greater cardiac output than the controls ($P<0.001$). Plasma sodium concentration was similar in the two groups. The hematocrit was lower ($P<0.001$) in the A-V rats ($39.5\pm0.89\%$) than in the control animals ($49.2\pm0.78\%$).

Acute saline loading resulted in a brisk natriuresis and diuresis in the normal rats ($P<0.001$), but was followed by only a minor increase in sodium and water excretion in A-V fistula rats ($P<0.001$). Total GFR rose significantly in both controls ($P<0.001$) and A-V fistula rats ($P<0.05$), while PAH clearance rose in control animals only ($P<0.01$). The small rise of this variable in the A-V rats was not statistically significant ($P>0.05$). Total filtration fraction was comparable in the two experimental groups, both before and after volume expansion.

Proximal micropuncture data. A summary of the micropuncture data is presented in Table 3, and the individual values for TF/P inulin are shown in Fig. 1. In A-V fistula rats, end-proximal TF/P inulin and the calculated fractional reabsorption of sodium and water up to the end of the proximal tubule were not significantly different from data obtained in normal rats. No significant difference between SNGFR for the two groups was found during hydropenia. Subsequently, the absolute rate of proximal reabsorption
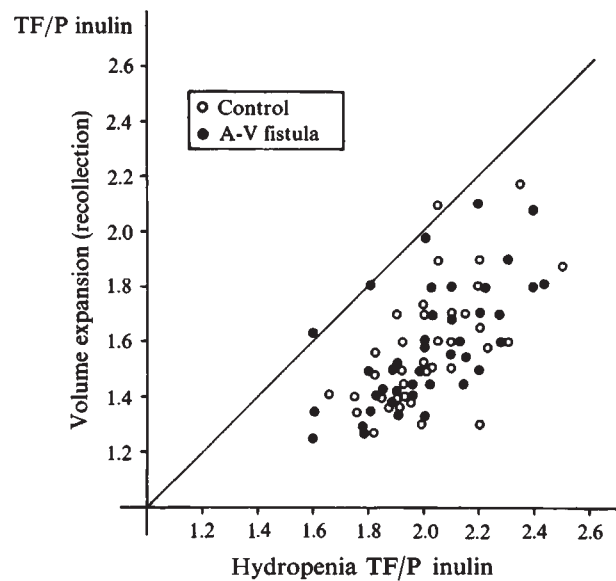

Fig. 1. *End-proximal TF/P inulin ratios in control and A-V fistula rats before and after extracellular fluid volume expansion.* Each *point* represents one paired recollection. *Points* below the *identity line* indicate a decrease in TF/P inulin respectively in fractional reabsorption.

and the rate of fluid delivery out of the proximal tubule in both control rats and A-V fistula rats were wholly comparable. Proximal tubular transit time was equal in the two groups. Following volume expansion, the fraction of filtrate reabsorbed up to the end-proximal puncture site decreased to the same extent in A-V fistula ($P<0.001$) and control animals ($P<0.001$), (Table 3 and Fig. 1). SNGFR rose significantly in the control rats ($P<0.01$); the small increase in the A-V rats was not significantly different ($P>0.05$). Absolute reabsorption by the proximal tubule fell significantly ($P<0.05$) in the A-V fistula rats, but decreased only slightly ($P>0.05$) in the control rats.

Table 3. Summary of proximal micropuncture data[a]

	Control rats		A-V fistula rats	
	Hydropenia ($N=12$)	Volume expansion ($N=12$)	Hydropenia ($N=14$)	Volume expansion ($N=14$)
TF/P inulin,[b] *nephrons*	2.00 ± 0.03 ($N=37$)	1.54 ± 0.03 ($N=37$)	2.02 ± 0.03 ($N=41$)	1.57 ± 0.04 ($N=41$)
Fractional reabsorption, %	50.0 ± 0.75	35.4 ± 1.35	50.0 ± 0.85	35.7 ± 1.33
SNGFR,[c] $\times 10^{-6}$ *ml/min/g kidney wt*	30.8 ± 1.25	39.8 ± 1.75	31.8 ± 2.01	35.6 ± 1.9
Filtrate reabsorbed, $\times 10^{-6}$ *ml/min/g kidney wt*	15.4 ± 0.68	13.8 ± 0.79	16.1 ± 1.01	12.9 ± 0.98
Proximal transit time, *seconds*	10.2 ± 0.25	7.4 ± 0.30	11.1 ± 0.35	7.8 ± 0.31

[a] Values are means $\pm$ SEM.
[b] TF/P = tubular fluid to plasma ratio.
[c] SNGFR = single nephron glomerular filtration rate.

Distal micropuncture data. Results of distal micropuncture data are presented in Table 4 and Fig. 2. The transit time of lissamine green through the loop of Henle during hydropenia was significantly slower in A-V fistula rats than in control rats ($P < 0.001$). A-V fistula rats had consistently higher early distal TF/P inulin ratios (4.95 ± 0.11) than the normal rats (3.81 ± 0.09), ($P < 0.001$). Subsequently, the fraction of filtrate left unreabsorbed at the early distal puncture site was lower ($P < 0.001$) in the A-V fistula rats than in control rats (Fig. 2). Early distal TF/P Na^+ ratio averaged 0.39 ± 0.02 in control rats and 0.32 ± 0.017 in A-V fistula rats ($P < 0.01$). The fraction of filtered sodium left unreabsorbed at the early distal puncture site was significantly lower in rats with an A-V fistula than in control rats ($P < 0.001$). Following volume expansion the early distal TF/P inulin ratio decreased to 2.20 ± 0.08 ($P < 0.001$) in control rats, and the TF/P Na^+ ratio rose to 0.44 ± 0.02, ($P < 0.05$). The corresponding TF/P inulin value for the A-V fistula rats was 4.3 ± 0.17 ($P < 0.05$) and the TF/P Na^+ ratio was 0.37 ± 0.019 ($P < 0.05$). From these values it can be calculated that during volume expansion the fraction of filtered sodium and water left unreabsorbed at the early distal puncture site was still significantly lower ($P < 0.001$) in the A-V fistula rats than in control rats (Fig. 2).

Intrarenal distribution of filtration. The individual values for single filtration rates of superficial, midcortical and juxtamedullary nephrons obtained with the aid of the modified Hanssen technique [13, 14] are detailed in Fig. 3. No significant difference in mean superficial, midcortical and juxtamedullary nephron GFR was found between controls and rats with an A-V fistula. Superficial and juxtamedullary nephron GFR measured by this technique
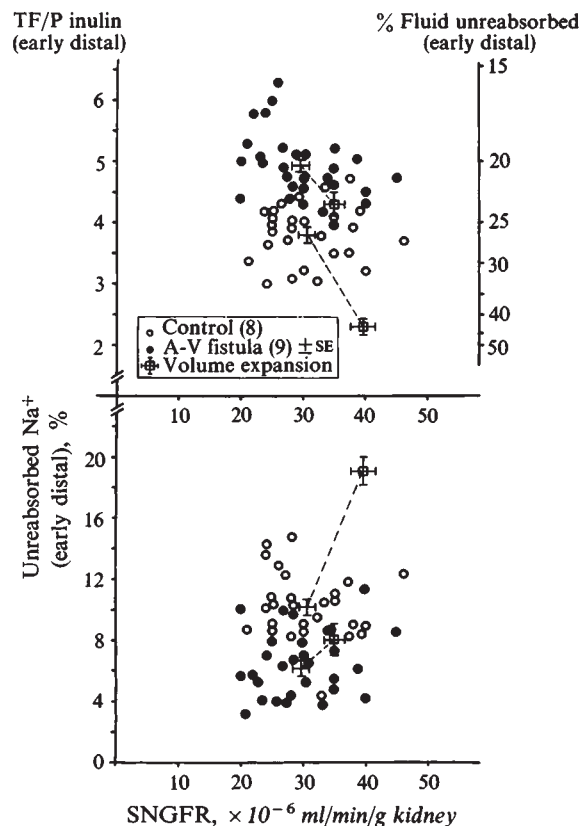

Fig. 2. *Relationship between SNGFR and unreabsorbed water (upper part) and sodium (lower part) remaining at early distal puncture site of the same nephron in control and A-V fistula rats.* The *points* plotted are preexpansion values. The *cross symbols* represent the mean values $\pm$ SEM. The *cross symbols with the open boxes* represent the mean values $\pm$ SEM obtained after extracellular fluid volume expansion.

Table 4. Summary of early distal micropuncture data[a]

	Control rats		A-V fistula rats	
	Hydropenia ($N=8$)	Volume expansion ($N=8$)	Hydropenia ($N=9$)	Volume expansion ($N=9$)
TF/P inulin *nephrons*	3.81 ± 0.09 ($N=27$)	2.20 ± 0.08 ($N=27$)	4.95 ± 0.11 ($N=29$)	4.30 ± 0.17 ($N=29$)
SNGFR[b] $\times 10^{-6}$ *ml/min/g kidney wt*	30.5 ± 1.18	39.5 ± 1.84	29.7 ± 1.24	35.2 ± 1.58
TF/P Na^+	0.39 ± 0.02	0.44 ± 0.02	0.32 ± 0.017	0.37 ± 0.019
[Na^+/inulin (TF/P)] $\times 100$, %[c]	10.3 ± 0.44	19.1 ± 0.85	6.3 ± 0.4	7.95 ± 0.48
Henle's loop transit time, *seconds*	25.5 ± 0.55	15.5 ± 0.38	32.5 ± 1.25	28.5 ± 1.15

[a] Values are means $\pm$ SEM.
[b] SNGFR = single nephron glomerular filtration rate.
[c] [Na^+/inulin (TF/P)] $\times$ 100 represents the fraction of filtered sodium left unreabsorbed at early distal puncture site.
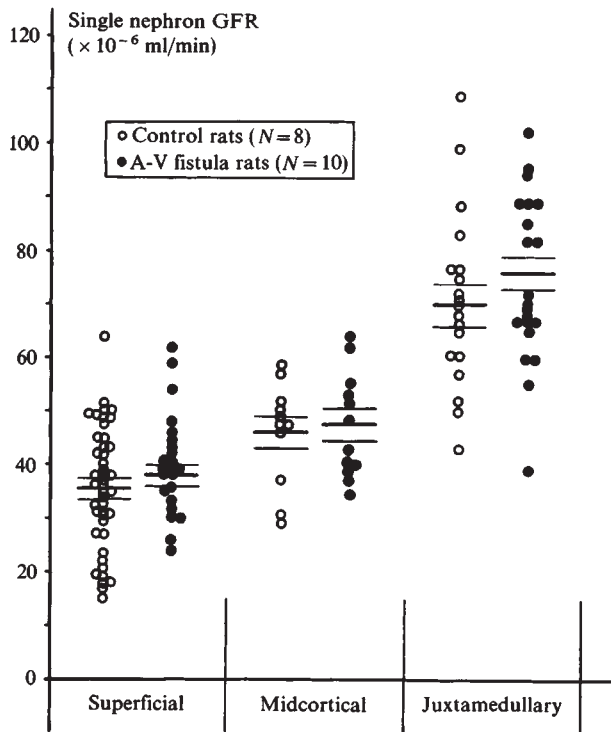
Fig. 3. *Intrarenal distribution of glomerular filtration in normal and A-V fistula rats.* Each *point* represents the average of 3 to 5 determinations. *Horizontal lines* represent the mean $\pm$ SEM.

was higher (approximately 20 per cent) than data obtained by micropuncture in both the present and previous work [10, 16].

Discussion

The present study demonstrates that rats with an A-V fistula can be used as a suitable model for studying chronic sodium retention and edema formation due to heart failure. In rats with an A-V fistula, sodium retention was not as severe as has been described for similar or other models of heart failure in dogs (3, 4, 6, 22]. This difference may be explained, in part, by the fact that A-V rats did not receive mineralocorticoid treatment following surgery. At the time of micropuncture, all the A-V rats were in a sodium-retaining stage. The limited ability of A-V fistula rats to excrete their daily sodium intake led within 14 days to the formation of peripheral edema, pleural effusions and ascites. These findings are very similar to those obtained in dogs [4–6] and humans [17–19] with high output heart failure due to a large arteriovenous fistula. However, unlike dogs with an A-V fistula in which low GFR and PAH clearance have been reported [6], the A-V fistula rats in this study had normal total kidney GFR and renal plasma flow. Since plasma sodium concentration was equal in controls and rats with an A-V fistula, the filtered load of sodium in the two groups was wholly comparable. A high

filtration fraction, a frequent finding in congestive heart failure, was not observed in rats with an A-V fistula.

It has been proposed that a redistribution of renal blood flow and glomerular filtration from superficial nephrons with low reabsorptive capacity to deeper and longer nephrons with higher capacity might be an important factor causing chronic salt retention in cardiac failure [8]. The data presented in Fig. 3 clearly demonstrate that a disproportionate decrease in superficial nephron GFR was not present in A-V fistula rats. Similarly, the ratio of superficial SNGFR (obtained by micropuncture) to total kidney GFR of both controls and A-V fistula rats was of the same order of magnitude. Thus, redistribution of filtration does not occur in A-V fistula rats and seems to be unnecessary for the development of chronic sodium retention. It is evident from this discussion that the decreased renal sodium excretion in the present model of cardiac failure shows no constant relationship to filtered loads of sodium, intrarenal distribution of filtrate, PAH clearance or filtration fraction.

From a study of dogs with heart failure due to the presence of an A-V fistula, Johnston et al [5] have suggested that an enhanced rate of sodium reabsorption by the proximal tubule might be important in the mechanism of sodium retention. Similarly, Cirksena, Dirks and Berliner [20] concluded from data obtained in acute caval dogs, another model of sustained salt retention, that the blunted natriuresis observed after saline loading in these dogs was mediated by increased proximal tubular reabsorption. The results presented in this report demonstrate that rats with an A-V fistula and chronic sodium retention have a normal proximal tubular function with regard to fractional and absolute sodium reabsorption. Moreover, the limited natriuretic response to acute saline loading in rats with an A-V fistula was not caused by a failure to decrease sodium reabsorption in the proximal tubule. Fractional sodium reabsorption by the proximal tubule fell to the same extent in A-V fistula rats as in control rats. However, after saline loading, it should be mentioned that A-V rats exhibited a much smaller increase in GFR and the filtered load of sodium than did control rats. Thus, the inability of the A-V rats to excrete a sodium load may be explained in part by a relatively fixed GFR. It is likewise possible that the blunting of natriuresis resulted from a lesser percent expansion of the extracellular space in A-V fistula rats.

The results on proximal tubular function presented in this report are very similar to micropuncture data obtained by Schneider et al [6] in dogs with an A-V fistula and by Levy [21] and Auld, Alexander and Levinsky [22] in chronic caval dogs. These authors also concluded that the proximal tubule of both chronic A-V dogs or chronic caval dogs functions normally with regard to sodium reabsorption. It cannot be excluded, however, that an enhanced rate of reabsorption by the proximal tubule may be present in the earliest stages of sodium retention and edema formation. The observations of Cirksena et al [20] in dogs with

acute caval constriction and subsequent increased proximal reabsorption bear on this point. Similarly, in a previous micropuncture study of A-V fistula rats, we observed an enhanced rate of sodium reabsorption by the proximal tubule 2 to 5 days after creation of the fistula [7]. At this time, changes in systemic and renal hemodynamics due to blood loss at the time of the operation might still have been present. It is known that acute reductions in renal hemodynamics, either by renal artery clamping [23] or extracellular fluid volume contraction [24], are accompanied by an increase in proximal fractional reabsorption. An alternative possibility might be that the immediate stimulus causing increased proximal sodium reabsorption in the A-V rats is still operating, but that subsequent depression of proximal tubule reabsorption by the expanded extracellular space has returned the net sodium absorption to the same as control. This would imply, however, that factors other than physical factors could mediate proximal tubule reabsorption. At present, the factors responsible for the early increase in proximal sodium reabsorption of A-V fistula rats are not entirely clear. Whatever these factors may be, this study has shown that they are probably not relevant to the chronic state of sodium retention in A-V fistula rats. At some as yet unknown point in time, proximal reabsorption of sodium in rats with an A-V fistula becomes normal and sodium reabsorption in a more distal nephron segment is enhanced. According to the present results, this nephron site is the loop of Henle. This conclusion is derived from the early distal micropuncture data obtained on superficial nephrons. During hydropenia, rats with an A-V fistula had significantly higher early distal TF/P inulin and lower Na^+/inulin (TF/P) ratios than control rats. Subsequently, the fraction of water and sodium left behind at the early distal puncture site was significantly lower in A-V rats than in control rats. Since the fractional and the absolute amount of fluid and sodium leaving the proximal tubule was equal in the two groups, reabsorption along the loop of Henle must have been enhanced in A-V rats. The finding of a significantly prolonged passage time of lissamine green through the loop of Henle also points to this nephron segment as one site of enhanced reabsorption. Moreover, during acute extracellular volume expansion, early distal TF/P inulin ratios were still higher and Na^+/inulin (TF/P) ratios were lower in A-V fistula rats than in control animals, indicating that water and sodium reabsorption by the loop remained increased under this experimental condition. These findings suggest that the loop of Henle plays a major role in the limited ability of the A-V rats to excrete sodium and water, during both hydropenia and saline loading. The present results do not exclude the possible role of the distal convoluted tubule and the collecting duct in salt retention of A-V fistula rats. Stein, Osgood and Ferris [25] have demonstrated that these nephron segments may be import in the final adjustment of sodium excretion during extracellular fluid volume expansion. Since in the present study no data were obtained

from late distal puncture sites, a segmental analysis of sodium reabsorption by the distal tubule and collecting duct is not possible.

It is interesting to note in A-V fistula rats that water reabsorption by the loop had increased to a greater extent than fractional sodium reabsorption. This is probably due to the different behavior of the loop. If, in the descending limb (including pars recta of the proximal tubule and the thin descending limb), some factor increases net water and sodium reabsorption to the same extent, less water and sodium would be delivered to the ascending limb. From the microperfusion study of Morgan and Berliner [26], it can be inferred that net reabsorption of sodium by the ascending limb increases at a higher load and decreases at lower loads. If this is accepted, and if the ascending limb is actually water impermeable, the difference in magnitude of sodium and water reabsorption would readily be explained.

The importance of enhanced distal sodium reabsorption in chronic salt retention due to heart failure has also recently been stressed by others [6, 21, 22]. Schneider, Dresser, Lynch and Knox [6] concluded from micropuncture data obtained in dogs with an A-V fistula that distal reabsorptive mechanisms play a major role in limiting the natriuretic response of these animals to acute volume expansion. Auld and Levinsky [22] reached a similar conclusion on the basis of micropuncture experiments performed in volume-expanded caval dogs. Recently, Levy provided evidence that the inability of chronic caval dogs to excrete a saline load is caused by an enhanced rate of reabsorption along the loop of Henle [21].

The mechanism whereby the loop of Henle appears to retain salt and water in A-V fistula rats is not clear. It has been demonstrated that an increase in aldosterone secretion is an essential element in the enhanced sodium reabsorption which occurs in dogs with an A-V fistula [4, 5]. However, normal dogs and rats return to sodium balance after only a few days of mineralocorticoid treatment, indicating that some other factor is also of importance in chronic salt retention [27]. Furthermore, in the rat mineralocorticoids have been shown not to affect the rate of sodium reabsorption by the loop of Henle, although they may enhance reabsorption by the distal convoluted tubule and the collecting duct [28, 29]. From the work of Davis, Howell and Hyatt [30], one may conclude that the kidney in congestive heart failure is more sensitive to mineralocorticoids than normally. Although the existence of an undefined humoral factor other than aldosterone [27] cannot be completely ruled out on the basis of available data, there is no conclusive evidence for its presence. Other workers have suggested that physical factors may play a role in the mechanism of salt retention due to experimental heart failure [21, 22, 31]. Friedler, Belleau, Martino and Earley [31] demonstrated that the diminished sodium excretion of chronic caval dogs during saline loading could be restored to normal when the low renal

artery pressure of these dogs was increased to normal levels. Similarly, Levy [21] observed a normal natriuretic response in his saline-loaded caval dogs after vasodilating one kidney and elevating the systemic arterial blood pressure. Moreover, Levy found that the elevation of renal perfusion pressure returned the enhanced rate of reabsorption by the loop to normal, indicating that altered intrarenal hemodynamics had probably influenced loop reabsorption. The observation that renal perfusion pressure was reduced in rats with an A-V fistula would suggest a change in intrarenal hemodynamics which might have influenced the rate of reabsorption by the loop. Thus, in view of the present findings and the observations of others discussed herein [21, 22, 31], it may be possible that the difference in sodium and water handling by the loop could be due to the differences observed in the blood pressure of the two groups. It has been demonstrated that in the rat acute and chronic changes in renal perfusion pressure may be accompanied by an altered rate of sodium reabsorption along the loop [10, 32] without the occurrence of alterations in PAH clearance and proximal tubule reabsorption [10, 33]. The normal PAH clearance of the A-V fistula rats does not exclude a change in intrarenal distribution of blood flow [33]. In the present study, no direct measurements of either intrarenal distribution of blood flow or of hydrostatic pressure in the vasa recta were obtained. Those data would be necessary to define the possible role of peritubular physical factors (such as a low hydrostatic peritubular capillary pressure) in the mechanism of enhanced reabsorption by Henle's loop observed in rats with an A-V fistula.

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